Leaf reflectance spectroscopy captures variation in carboxylation capacity across species, canopy environment and leaf age in lowland moist tropical forests

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Summary

- Understanding the pronounced seasonal and spatial variation in leaf carboxylation capacity ($V_{c,max}$) is critical for determining terrestrial carbon cycling in tropical forests. However, an efficient and scalable approach for predicting $V_{c,max}$ is still lacking.
- Here the ability of leaf spectroscopy for rapid estimation of $V_{c,max}$ was tested. $V_{c,max}$ was estimated using traditional gas exchange methods, and measured reflectance spectra and leaf age in leaves sampled from tropical forests in Panama and Brazil. These data were used to build a model to predict $V_{c,max}$ from leaf spectra.
- The results demonstrated that leaf spectroscopy accurately predicts $V_{c,max}$ of mature leaves in Panamanian tropical forests ($R^2 = 0.90$). However, this single-age model required recalibration when applied to broader leaf demographic classes (i.e. immature leaves). Combined use of spectroscopy models for $V_{c,max}$ and leaf age enabled construction of the $V_{c,max}$–age relationship solely from leaf spectra, which agreed with field observations. This suggests that the spectroscopy technique can capture the seasonal variability in $V_{c,max}$, assuming sufficient sampling across diverse species, leaf ages and canopy environments.
- This finding will aid development of remote sensing approaches that can be used to characterize $V_{c,max}$ in moist tropical forests and enable an efficient means to parameterize and evaluate terrestrial biosphere models.

Introduction

Projecting the fate of terrestrial ecosystems under a changing climate requires knowledge of plant physiology and ecology, and representation of that process knowledge in Earth system models (ESMs). Photosynthesis is an especially critical process to represent accurately. In the most widely used model of photosynthesis, the rate of CO_2 assimilation is determined by the maximum carboxylation rate of the enzyme Rubisco ($V_{c,max}$), the rate of RuBP regeneration through electron transport, and in some models, the utilization of triose phosphates (Farquhar *et al.*, 1980; Sharkey *et al.*, 2007). The $V_{c,max25}$, which is $V_{c,max}$ standardized to a reference temperature of 25°C (Bernacchi *et al.*, 2013), is a key parameter at the heart of many ESMs, and variation in this parameter has repeatedly been shown to be the source of a large fraction of overall model uncertainty (Bonan *et al.*, 2011; Rogers, 2014; Rogers *et al.*, 2017a; Walker *et al.*, 2017; Ricciuto *et al.*, 2018). Accurate and comprehensive observations of the biogeography, ecology and overall distribution of $V_{c,max25}$ are thus a

critical research need for improving understanding of and model predictions for photosynthesis at local, regional and global scales.

Most ESMs currently represent $V_{c,max25}$ with a single static value for each plant functional type (Bonan *et al.*, 2011; Rogers, 2014). This assumption is most questionable for the tropical forest biome where forests hold enormous plant functional diversity (Condit *et al.*, 2005; Steege *et al.*, 2013; Asner *et al.*, 2014) that includes diversity in photosynthetic capacity (Norby *et al.*, 2017; Walker *et al.*, 2017). Furthermore, for a given species, $V_{c,max25}$ has been shown to vary greatly with leaf development, growth temperature, and water and nutrient availability (Medlyn *et al.*, 1999; Wilson *et al.*, 2001; Kenzo *et al.*, 2006; Kattge & Knorr, 2007; Ali *et al.*, 2015; Norby *et al.*, 2017; Albert *et al.*, 2018; Kumarathunge *et al.*, 2019; Smith *et al.*, 2019). Recently it was shown that the seasonality of photosynthesis in Amazonian evergreen forests, a *c.* 4 Gt yr^{-1} fluctuation in CO_2 assimilation (estimated using the envelop calculation approach to extend existing site-level study in Amazon to the entire Amazon basin), is driven by the replacement of old leaves that have a low $V_{c,max25}$

with recently matured leaves that have a higher $V_{c,max25}$ (Wu *et al.*, 2016; Albert *et al.*, 2018). These studies also demonstrated that it is critical to quantify leaf age and couple this information with estimates of $V_{c,max25}$ to more accurately model leaf CO_2 assimilation by tropical forests. This result is likely also to be applicable to other vegetative biomes that contain plants with long-lived leaves (e.g. needle-leaf evergreen) or with significant seasonal variation (Wilson *et al.*, 2001; Han *et al.*, 2008; Muraoka *et al.*, 2010; Niinemets, 2016). However, scaleable $V_{c,max25}$ data to enable this approach in models is lacking and tedious to collect in the tropics, which is reflected in the very poor geographical coverage of tropical plants in plant trait databases (Kattge *et al.*, 2011; Schimel *et al.*, 2015; Díaz *et al.*, 2016).

Typically, leaf-level $V_{c,max25}$ is estimated by fitting a model to a photosynthetic CO_2 response curve measured using gas exchange in a process that can take over 45 min for a single measurement (Long & Bernacchi, 2003). Although faster methods of estimating $V_{c,max}$ have been described recently (De Kauwe *et al.*, 2016; Stinziano *et al.*, 2017), gas exchange measurements remain challenging in natural systems such as tropical forests where many species must be characterized at large scales. Canopy access presents an additional challenge in some systems, including tropical forests where canopy height can exceed 30 m, requiring canopy cranes or tree climbing, which may be prohibitively time-consuming or expensive. Moreover, reliably tracking leaf age – using the leaf tagging method with intensive *in situ* revisits and surveys (Reich *et al.*, 2004; Wu *et al.*, 2017) – coupled with leaf gas exchange measurements, adds another level of difficulty. This challenge is particularly acute for moist tropical forests in which periods of new leaf production can last from a week up to a year, and different tree species have distinct and often irregular new leaf production patterns both in their timing and amplitude (Reich *et al.*, 2004; Lopes *et al.*, 2016; Xu *et al.*, 2017). Within this context, researchers require methods that allow rapid estimation of $V_{c,max25}$ and leaf age that can be applied to tall trees in natural systems, including remote tropical forests.

Recent advances in vegetation spectroscopy offer a promising solution given that this approach tightly connects leaf optical properties with their chemical composition, cell structure and physiological properties (Curran, 1989; Elvidge, 1990; Kokaly *et al.*, 2009). As such, spectroscopy has been receiving increasing attention from a broader science community, including those from plant ecophysiology, functional trait ecology and evolution (Serbin *et al.*, 2012; Asner *et al.*, 2016; Schneider *et al.*, 2017; Schweiger *et al.*, 2018). For example, recent studies suggest that leaf $V_{c,max}$ can be estimated accurately and rapidly based on leaf reflectance spectra (Doughty *et al.*, 2011; Serbin *et al.*, 2012; Ainsworth *et al.*, 2014; Barnes *et al.*, 2017; Dechant *et al.*, 2017; Yendrek *et al.*, 2017; Silva-Perez *et al.*, 2018). In addition, two recent studies also have shown that leaf spectroscopy provides an accurate, rapid means to assess leaf age at both individual and community scales (Chavana-Bryant *et al.*, 2017; Wu *et al.*, 2017). Furthermore, some studies also suggest that it is possible that spectroscopy-based models of leaf $V_{c,max25}$ and age could be extended to the canopy scale by leveraging imaging spectroscopy

instrumentation on tower, unmanned aerial systems and manned airborne platforms (Serbin *et al.*, 2015; De Moura *et al.*, 2017). These developments highlight the potential to map changes in $V_{c,max25}$ and leaf age over unprecedented spatial and temporal scales. However, the ability of spectra to predict the variability in $V_{c,max25}$ across these multiple axes of variation (i.e. species, canopy position, leaf age and forest sites) has not been tested, and a spectroscopy-based approach that can account for variation in both leaf age and $V_{c,max25}$ has not been developed.

In the present study leaf gas exchange, reflectance spectroscopy and leaf age data were collected from three lowland moist tropical forests. Our goal was to develop a single spectroscopic approach capable of capturing the variation in $V_{c,max25}$ among leaves of different ages from a range of species and canopy environments (i.e. variation in canopy height and sunlit and shaded environments) in lowland moist tropical forests. Two main questions were addressed: (1) Can the spectra–$V_{c,max25}$ relationship for mature leaves also be applied to leaves of other leaf demographic classes (e.g. immature leaves), and if not, can a new spectra-based model of $V_{c,max25}$ be developed that performs well across all leaf ages? (2) Can leaf spectra information alone enable accurate estimation of the developmental trajectories of $V_{c,max25}$: the $V_{c,max25}$–leaf age relationship? By answering these questions, the hope was to understand if the spectroscopy approach can be used to capture the $V_{c,max25}$ variability in moist tropical forests, thereby accelerating current capacity to parameterize ESMs for improved projection of terrestrial carbon and water fluxes in the context of a changing climate.

Materials and Methods

Site descriptions

This study used data collected from three lowland seasonal moist tropical forests, including two crane sites in the Republic of Panama and one site in Brazil. The two sites in Panama include a seasonally dry forest in the Parque Natural Metropolitano (PNM; 8.9950°N, 79.5431°W) near Panama City and a wet evergreen forest in the San Lorenzo Protected Area (SLZ; 9.2810°N, 79.9745°W), Colon Province. Both sites are dominated by clay soil (Turner & Romero, 2009). Mean annual air temperature at both sites is 26°C (1998–2015), and mean annual precipitation is 1826 and 3286 mm yr^{-1} for PNM and SLZ, respectively, with a 4-month-long dry season (precipitation < 100 mm per month) from January to April each year. At each site, the Smithsonian Tropical Research Institute maintains a canopy-access crane that enables access throughout the canopy of these forests. The site in Brazil (2.8500°S, 54.9667°W) is located around the K67 eddy covariance site in Tapajos National Forest, near Santarem, Para, Brazil. Part of the Brazilian Large Scale Biosphere-Atmosphere Experiment in Amazonia (LBA; Davidson *et al.*, 2012), this site sits on a well-drained clay-soil plateau. Multiple-year (2002–2005) mean annual air temperature is 26°C (Hutyra *et al.*, 2007). Mean annual precipitation (1998–2013) is 2022 mm yr^{-1} with a 5-month-long dry season from mid-July to mid-December each year. Single rope access

techniques were used to climb into and access individual crowns of canopy trees (Albert *et al.*, 2018). For details about forest composition and structure of the sites in Panama see Wright *et al.* (2003), and of the site in Brazil see Rice *et al.* (2004). For information about soil fertility of the sites in Panama see Turner & Romero (2009), and of the site in Brazil see Nepstad *et al.* (2002).

Plant materials

Sixteen canopy tree species from the two sites in Panama ($n = 8$ for SLZ and $n = 8$ for PNM) and five canopy tree species from the Brazilian site were selected for intensive field measurements of leaf gas exchange, reflectance spectra and traits (i.e. leaf mass per area, LMA; Supporting Information Table S1). Sampled leaves were classified into two main age classes: immature leaves (< 2 months; corresponding to the leaves from emergence up to fully expanded, but not fully green, thickened, or physiologically matured) or mature leaves (≥ 2 months old), following the similar age categories as presented in Coley (1983), Wu *et al.* (2016), and Albert *et al.* (2018). This classification of leaf age is very similar to the three-age-category (young, mature and old) used in Wu *et al.* (2016), except that mature and old age classes were grouped together into a single mature age class, the reason being that leaf age was not tracked as frequently in Panama as in Brazil (Wu *et al.*, 2017), and therefore lacked the resolution to differentiate three age classes. Field measurements in Panama were conducted in the 2016 and 2017 dry seasons on sunlit upper canopy foliage. In the 2016 field campaigns in mid-February and mid-April the dominant leaf class(es) were sampled from eight trees at each site. In February 2017 the measurements included both age classes if present within the top meter of a sunlit branch from four canopy tree species at the SLZ site (Table S1). Field measurements of canopy trees in Brazil, including leaves of both age classes from sunlit and shaded branches, were conducted during the 2012 dry season field campaign from mid-August until early-December and a 2013 dry season campaign in August, using single-rope access techniques. For more details on surveyed tree species, please refer to Table S1 and Albert *et al.* (2018).

Field measurements

Leaf gas exchange Six portable gas exchange systems were used in Panama and two in Brazil (LI-6400XT; Li-COR Inc., Lincoln, NE, USA). Measurements of the response of net assimilation rate (A) to intracellular CO_2 concentration (C_i), commonly known as A–C_i response curves, were conducted on leaves from cut branches. In Panama, all branches were sampled before dawn using the canopy cranes. Steps were taken to avoid inducing xylem embolism when collecting branches, and when it was possible, the initial cut was made under water by bending the branch section into a bucket filled with water. Otherwise, the initial cut was made in the air and then a second cut was made underwater *c.* 1 m from the initial cut. In all cases, several cuts were made sequentially closer to the branch tip to relax xylem tension, following the protocol as described by Sperry (2013). Samples were stored in individual buckets and kept in deep shade until used for measurements, which was normally within 4 h after harvesting. Measurements of A–C_i curves closely followed Rogers *et al.* (2017b), with the reference CO_2 concentration controlled as follows: 400, 325, 250, 175, 100, 65, 40, 400, 400, 400, 475, 575, 675, 800, 1000, 1400 and 1800 $\mu mol\ mol^{-1}$; leaves were held at $31 \pm 2°C$ and $83 \pm 5\%$ relative humidity under saturated light conditions (i.e. 2000 $\mu mol\ m^{-2}\ s^{-1}$). In Brazil, branch samples were collected via tree climbing, and gently lowered to the ground with ropes in the morning, and re-cut under water within 15 min. Samples were stored in individual buckets and kept in deep shade until used for measurements (typically within 4 h after harvesting). Full details of leaf gas exchange measurements from Brazil are in Albert *et al.* (2018). In brief, the protocol was similar to that described above except that in Brazil, the reference CO_2 concentration was controlled as follows: 400, 100, 50, 100, 150, 250, 350, 550, 750 $\mu mol\ mol^{-1}$, and then increased by increments of between 200 and 500 to reach saturation at *c.* 2000; leaf temperature was controlled at $31 \pm 2°C$ and chamber humidity was controlled at $46 \pm 11\%$.

Before curve-fitting, quality control procedures for gas exchange measurements from all sites excluded values associated with instrument error and other known artifacts, such as spurious logs and data where leaks were clearly apparent, as described in Rogers *et al.* (2017b) and Albert *et al.* (2018). Finally, apparent maximum carboxylation capacity standardized to a reference temperature of 25°C ($V_{c,max25}$) was estimated using the kinetic constants and temperature response functions presented by Bernacchi *et al.* (2013) as described by Rogers *et al.* (2017b). A total of 186 leaves with estimated $V_{c,max25}$ in Panama and 81 leaves in Brazil were used in the present study, with species-specific mean and standard deviation summarized in Table S1.

Leaf spectra Following leaf gas exchange measurements, the branches were kept in water and within 2 h harvested the leaf and immediately measured leaf reflectance spectra and fresh mass. Leaf reflectance at the Panamanian sites was measured using a Spectra Vista Corporation (SVC) HR-1024i (SVC, Poughkeepsie, NY, USA; spectral range: 350–2500 nm; spectral resolution: 3.5 nm at 700 nm, 9.5 nm at 1500 nm, and 6.5 nm at 2100 nm) together with the SVC LC-RP-Pro foreoptic. Similarly, leaf reflectance of Brazilian plants was measured using a FieldSpec® Pro spectrometer (Analytical Spectra Devices (ASD), Boulder, CO, USA; spectral range: 350–2500 nm; spectral resolution: 3 nm at 700 nm, 10 nm at 1400 nm, and 10 nm at 2100 nm) together with an ASD leaf clip attached to a plant probe assembly. In both cases, the reflectance probes contained internal, calibrated light sources to illuminate the samples during spectral collection. The leaf probe was used together with a black background for leaf reflectance measurements. To avoid the excessive heat loads while ensuring the reliable spectral collection, the ASD integration time was set to 100 ms per scan and each collected spectra was an average of 10 scans, whereas with the SVC a 1 s collection time was used with the spectrometer's automatic integration optimization. This approach matches that of Serbin *et al.* (2012), which originally highlighted the concerns of the excessive

heat loads on the data quality of leaf spectra collected. For each leaf, reflectance spectra were measured on 1–6 different parts of the leaf adaxial surface depending on leaf size, and then averaged to determine the mean optical properties across all wavelengths.

Leaf traits Leaf mass per area (LMA; g m^{-2}) also was measured to assess the diversity of plant species that were sampled in terms of the LMA trait space. In Panama, a known leaf area was sampled using cork borers. The samples were dried to constant mass at 70°C. Dry mass was then determined with a precision balance (Model SLF303; Fisher Science Education, Hanover Park, IL, USA) to calculate LMA. In Brazil, LMA was derived from area (using a Canon LiDE 120 scanner; Canon USA Inc., Melville, NY, USA) and DW (also using a precision balance from Fisher Science Education, Model SLF303, Hanover Park, IL, USA) oven-dried at 60°C for over 72 h.

Leaf age In Brazil, in field campaigns conducted in August–September 2013, November 2013, March 2014 and July–August 2014, seven trees of different species were selected (see Table 1 in Wu *et al.*, 2017) for precise *in situ* leaf age monitoring. Leaf age monitoring was carried out by using metal tags and *in situ* photo documentation (e.g. fig. S1 in Wu *et al.*, 2017). Monitoring began in August–September 2013, when most sampled trees were flushing new leaves, and was continued periodically throughout the annual cycle. Through this age-tagging technique, leaf age was accurately tracked from leaf emergence at budburst (0 d) up to *c.* 400 d old. From those leaves with accurate leaf age monitoring, a total of 759 leaves was then sampled covering the entire annual cycle, and measured leaf reflectance spectra using the same ASD FieldSpec® Pro spectrometer as described above. These leaves were then used for the development of the community-level spectra-age model (Wu *et al.*, 2017) and briefly summarized below. Among these seven trees surveyed for both leaf age monitoring and leaf reflectance measurements, four were the same canopy trees (including leaf samples from both sunlit and shaded microenvironments) from which were made gas exchange measurements as described above (also see Table S1).

In addition to the above-mentioned accurate leaf age monitoring, RGB photos were also taken for all leaves used for leaf reflectance measurements in both Panama and Brazil. These RGB photos together with other related information, for example visual assessment of the color, size and rigidity of the leaves, and relative positions and bud scars (when present) within a 1-m branch length, were then used to classify these leaves into two different age categories: immature and mature leaves.

Spectra–$V_{c,max25}$ analysis For all field-based spectral and gas exchange measurements in Panama and Brazil, only the plant species with both leaf reflectance spectra and $V_{c,max25}$ were selected (which excluded 25 measurements in Panama and 12 measurements in Brazil with only leaf gas exchange). Finally, a filtering was performed of outliers of combined spectra–$V_{c,max25}$ datasets (which removed *c.* 5% of data). The outlier detection method implemented here was originally used in Wu *et al.* (2017), which adapted an outlier detection module from 'LIBPLS'

(accessed at https://www.libpls.net/), using the Monte Carlo sampling method (Xu & Liang, 2001) for automatic outlier detection. After the data filtering, the Panamanian dataset had $n = 151$ measurements, including 110 mature leaves from all 16 species and 41 immature leaves from nine of the 16 species, which accounts for 94% of all measurements with both spectral and leaf gas exchange in Panama. The Brazilian dataset had $n = 65$ measurements, including 44 mature leaves and 21 immature leaves from all five species, which accounts for 94% of all measurements with both spectral and leaf gas exchange in Brazil.

All of the data sources associated with this study including gas exchange data, leaf spectral data, leaf trait and leaf age information are summarized in Table S2.

Partial least-squares regression (PLSR) modeling of spectra–$V_{c,max25}$ and spectra-age

In order to relate the variability in $V_{c,max25}$ across tree species, leaf age, canopy position and forest sites with the variability in leaf reflectance spectra and to infer leaf age from leaf reflectance spectra, a PLSR modeling approach (Geladi & Kowalski, 1986; Wolter *et al.*, 2008) was utilized using the 'plsregress' function in MATLAB (Mathworks, Natick, MA, USA) as described in De Jong (1993) and Rosipal & Krämer (2006). PLSR is a commonly used approach in spectroscopy and chemometric analyses given its ability to handle high predictor collinearity and a large number of predictor variables that may exceed the number of observations. PLSR accounts for these challenges by reducing the number of predictor variables down to relatively few, orthogonal latent variables, each composed of a weighted sum of the original variables (Geladi & Kowalski, 1986; Wolter *et al.*, 2008). Moreover, PLSR accounts for measurement error in the predictor variables (i.e. leaf hyperspectral reflectance).

The PLSR model development used herein has been described previously (Wu *et al.*, 2017) and is briefly summarized here. First a square-root transformation was applied to the $V_{c,max25}$ data and leaf age data to reduce the right skewness distribution of the original data (e.g. Figs S1a,b, S2a,b) and satisfy the normal distribution assumption of PLSR analysis. Then a one-time, random, stratified separation of the full dataset into calibration (two-thirds) and independent validation (one-third) subsets was performed; stratification ensured that each subset included leaf samples of each age category, of each species, and (when appropriate) of each canopy position. Next, 70% of the calibration data subset was randomly selected and used to fit the PLSR model of spectra–$V_{c,max25}$; this was repeated 100 times and for each permutation applying the model to predict the corresponding independent 30% of the calibration data. To avoid the potential to over-fit the spectra-based calibration model, the number of PLSR latent variables was optimized by choosing the number of latent variables that minimized the root mean square error (RMSE) from predicting the remaining 30% of the calibration data over the 100 permutations (Chen *et al.*, 2004; e.g. Fig. S1c). Meanwhile, the mean and standard deviation also were determined for the distribution of PLSR coefficients generated by the 100 PLSR fits corresponding to the optimal number of latent

variables, which were used in the final spectra–$V_{c,max25}$ model (e.g. Fig. S1d).

Finally, the performance of this spectra–$V_{c,max25}$ model was quantified using the independent validation dataset. Three main evaluation metrics were used: the coefficients of determination (R^2), RMSE and the regression bias. All model results presented in this study are shown in the original $V_{c,max25}$ units rather than the square-root transformed unit that is the initial output of the PLSR model. The same spectral analytical approach was applied to the Brazilian spectra-age dataset to derive the community-level spectra-age model (Fig. S2 and Wu *et al.*, 2017). All of the code used for model development and data analysis were developed in MATLAB (Mathworks).

Generalizability of spectra–$V_{c,max25}$ relationship

In order to determine whether the spectra–$V_{c,max25}$ relationship can be generalized across species, leaf age and canopy environment two tests were performed. In the first test, a spectra-based PLSR model was developed using two-thirds of the Panamanian data for mature leaves to train the model (including all 16 species). This model was then applied to the remaining Panamanian dataset of mature leaves, as well as to the independent validation dataset of Panamanian immature leaves, and Brazilian mature and immature leaves. Through this test, it would enable assessment of whether the spectra–$V_{c,max25}$ relationship of mature leaves also can be applied to leaves of immature age class or leaves of a different forest site in Brazil. In the second test, a new spectra-based model was developed in which all datasets (including two-thirds of all leaves from both Panama and Brazil) were used to train the model. Model performance was evaluated using the remaining, independent datasets, which are the same as that were used in the first test. Through this test, it would enable assessment of whether a single spectra–$V_{c,max25}$ relationship can be applied to leaves of both leaf age classes and different forest sites.

Spectral-based seasonal variability in $V_{c,max25}$, or $V_{c,max25}$–age relationship, using a combination of spectral models of $V_{c,max25}$ and leaf ae

With the developed spectra–$V_{c,max25}$ model and spectra-age model as described above, these two models were then used in combination to explore whether leaf spectra information alone can be used to reconstruct the life-history variability in leaf $V_{c,max25}$ for tropical trees. The spectra-age dataset in Brazil were used for this test, as the dataset covered the leaf spectra throughout their life cycles (Wu *et al.*, 2017) while having some ground truth of $V_{c,max25}$ derived from gas exchange measurements (Albert *et al.*, 2018). These two models are both at the community level and their model regression coefficients are shown in Figs S1(d) and S2(d), respectively. The models were driven by the input of leaf spectral reflectance only but can predict leaf $V_{c,max25}$ and leaf age, respectively (see the provided sample MATLAB script in Notes S1). By combining the model output of $V_{c,max25}$ and age together, the spectroscopy approach was thus used to estimate the $V_{c,max25}$–age relationship, or the life-history variability in leaf $V_{c,max25}$ with leaf age.

Results

As shown in Fig. 1 and Table S1, large variability in leaf $V_{c,max25}$ was found for the surveyed 21 tropical trees from three tropical
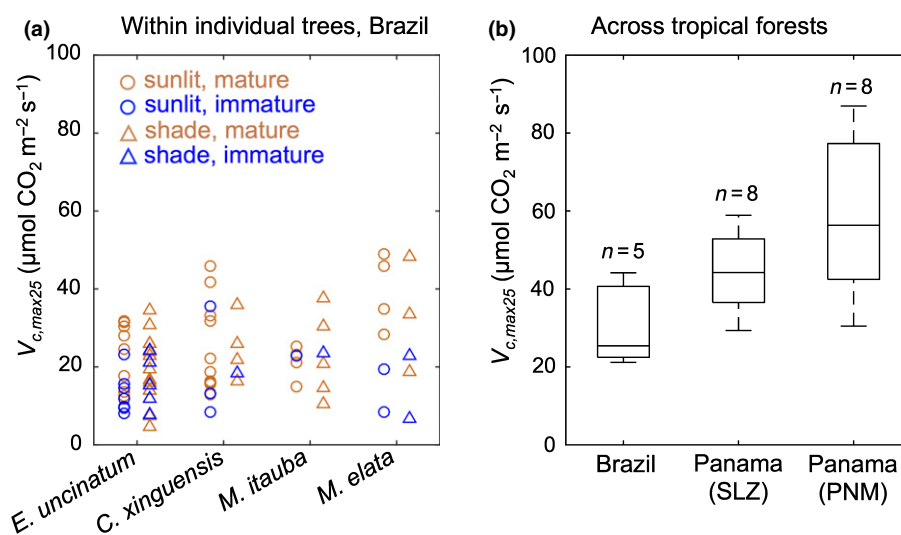

Fig. 1 Large variation in leaf maximum carboxylation rate of Rubisco standardized to 25°C ($V_{c,max25}$) (a) within individual trees and (b) across tropical forests. The data were from the Tapajos K67 site in Brazil, the San Lorenzo crane site (SLZ) and the Parque Natural Metropolitano crane site (PNM) in The Republic of Panama. At the individual tree level (a), $V_{c,max25}$ is associated primarily with leaf development (orange for variation in mature leaves, and blue for variation in immature leaves; see the Materials and Methods section) and canopy position (circles for the leaves sampled from the sunlit canopy environment, and triangles for the leaves sampled from the shaded canopy environment). Across tropical forests (b), the spread of tree specific (sunlit canopy, mature leaves) mean $V_{c,max25}$ of each forest site is displayed with a boxplot, in which the central mark is the median, the edges are the 25th and 75th percentiles, and the whiskers are the 5th and 95th percentiles.

forest sites: field-measured $V_{c,max25}$ ranged from 7 to 102 µmol CO_2 m^{-2} s^{-1}. These surveyed trees also spanned a very large variation in leaf morphology, as shown in the observed LMA trait space (i.e. 70–213 g m^{-2}). It was also found that the variation in $V_{c,max25}$ is attributable to species (Fig. 1a; Table S1), leaf age (mature vs immature; Fig. 1a), canopy environment (sunlit vs shaded; Fig. 1a) and forest sites (Fig. 1b). The large intraspecific variation in $V_{c,max25}$ is primarily associated with leaf age, and the large interspecific variation in $V_{c,max25}$ is attributable to both species difference and forest sites. Such large variation in $V_{c,max25}$, especially the variation with leaf age, will make the traditional approaches for measuring this diversity challenging due to the requirement for lots of measurements.

The first question of whether the spectroscopy approach can be an efficient, alternative means to help estimate $V_{c,max25}$ across species, leaf age, canopy environment and forest sites was evaluated through the two tests (see the Materials and Methods section above). In the first test, it was found that the model based solely on Panamanian mature leaves was able to predict the field-observed $V_{c,max25}$ of independent Panamanian mature leaves with very high accuracy ($R^2 = 0.90$; RMSE = 5.9 µmol CO_2 m^{-2} s^{-1}; $n = 36$ leaves; Fig. 2a), suggesting a tight covariation in the spectra–$V_{c,max25}$ relationship for Panamanian forests across a diverse range of tree species, canopy heights and leaf traits (Table S1). However, this model did not perform as well when applied to a dataset of independent, Panamanian immature leaves from a subset of the same trees ($n = 9$ species; Table S1), with the model fit having a marked deviation from the 1 : 1 line (between modeled and observed $V_{c,max25}$) and displaying poor predictive ability ($R^2 = 0.02$; RMSE = 20.7 µmol CO_2 m^{-2} s^{-1}; $n = 14$ leaves; Fig. 2b). When the model of Panamanian mature leaves was

applied to independent, Brazilian mature and immature leaves the model performance also was poor ($R^2 = 0.23$; RMSE = 38.8 µmol CO_2 m^{-2} s^{-1}; $n = 22$ leaves; Fig. 2c). This showed that the model developed from Panamanian mature leaves could be directly applied neither to immature leaves of the same trees nor leaves sampled from other tropical forests without marked reduction in predictive power (Fig. 2d).

In the second test, it was found that the new model trained on the data from all species, leaf ages, canopy environment and forest sites performed dramatically better across the whole range of leaf types – immature leaves in Panama ($R^2 = 0.89$; RMSE = 3.9 µmol CO_2 m^{-2} s^{-1}; $n = 14$ leaves; Figs 3, S3a) and all leaf ages from Brazil ($R^2 = 0.68$; RMSE = 5.9 µmol CO_2 m^{-2} s^{-1}; $n = 22$ leaves; Figs 3, S3b) – at the cost of only slightly lower prediction of mature Panamanian leaves ($R^2 = 0.86$; RMSE = 7.7 µmol CO_2 m^{-2} s^{-1}; $n = 36$ leaves; Figs 3, S3c). In addition, compared with the initial model of Panamanian mature leaves (Fig. 2, first test), the new model (Fig. 3, second test) also significantly reduced the uncertainty in model predicted $V_{c,max25}$, as indicated by the horizontal error bars shown in Figs 2 and 3. This analysis demonstrated that with sufficient leaf samples to train the spectra–$V_{c,max25}$ relationship over the full trait space, a general spectra-based $V_{c,max25}$ model can be derived across species, leaf age, canopy environment and forest sites.

The second question of whether the spectroscopy approach alone is sufficient to simulate the life-history trajectories of $V_{c,max25}$, or the $V_{c,max25}$–age relationship was then examined. To do this, two models (i.e. the community-level spectra–$V_{c,max25}$ model and spectra-age model) were applied to the spectra-age dataset in Brazil (see the Materials and Methods section). The
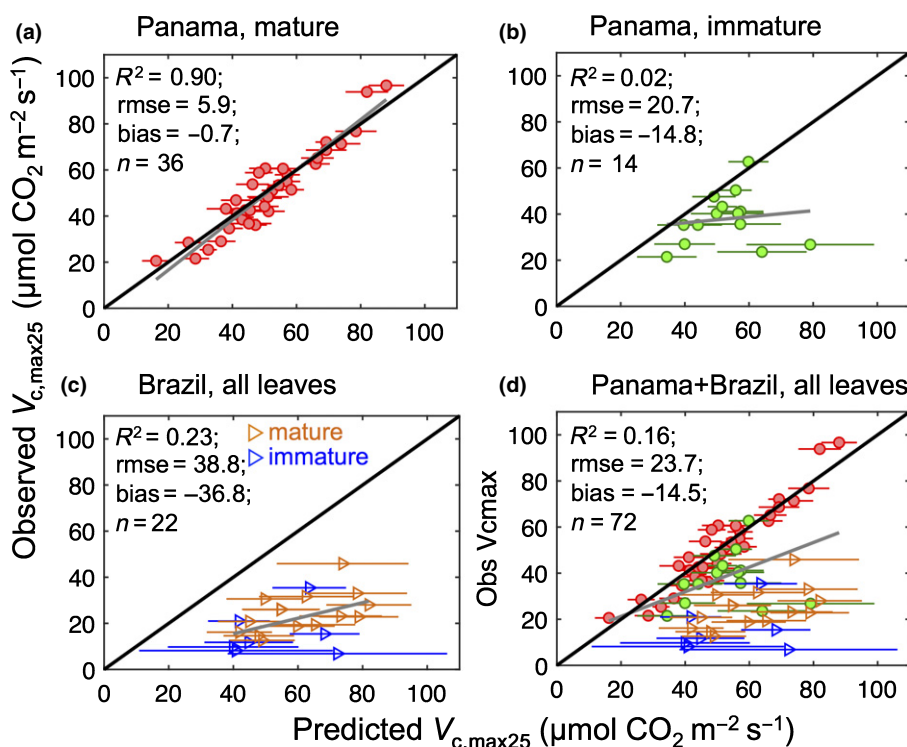
Fig. 2 A spectra–$V_{c,max25}$ model was trained using two-thirds of the dataset from mature leaves measured in Panama, and then evaluated using the independent validation dataset collected in (a) Panamanian mature leaves, (b) Panamanian immature leaves, (c) Brazilian mature and immature leaves, and (d) all leaf classes collected in Panama and Brazil. Error bars denote the 95% confidence intervals for each predicted value based on the ensemble partial least-squares regression (PLSR) models (i.e. each PLSR model is represented by a set of PLSR fitted spectral coefficients, and in total includes 100 Monte Carlo model runs; see the Materials and Methods section); the gray line shows the ordinary least-squares regression fit, and the black line shows the 1 : 1 line. $V_{c,max25}$, maximum carboxylation rate of Rubisco standardized to 25°C.

Fig. 3 The final spectra–$V_{c,max25}$ model was trained using two-thirds of our entire dataset, and then applied to the remaining, independent validation dataset. Error bars denote the 95% confidence intervals for each predicted value based on the ensemble PLSR models, the gray line shows the ordinary least-squares regression fit, and the black line shows the 1 : 1 line. $V_{c,max25}$, maximum carboxylation rate of Rubisco standardized to 25°C.
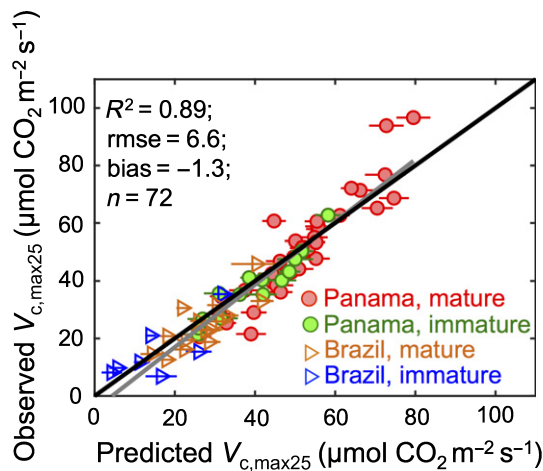
results showed that there was large variability in leaf $V_{c,max25}$ across leaf life cycles (i.e. from 15 to 400-d old), but that the spectroscopy approach presented here was able to track leaf age-dependent variation in $V_{c,max25}$ (Fig. 4), particularly during the period from emergence to physiological full maturity (from 15 to 150 d). With continued aging and senescence (> 150 d), there was larger deviation between spectra-predicted and field-observed $V_{c,max25}$. This might largely be because the datasets herein had poor coverage of older leaves (from 150 to 400 d), and thus the model for the senescent leaves is not well calibrated.

Discussion

Leaf carboxylation capacity $V_{c,max25}$ (the maximum carboxylation rate of the enzyme Rubisco standardized to a reference temperature of 25°C) is central to the estimation of photosynthetic CO_2 uptake by tropical forests in Earth system models (ESMs). In the present study, it was demonstrated that the spectroscopy approach is able to accurately predict $V_{c,max25}$ across species with a large range in leaf mass per area (LMA) trait space, leaf age, canopy position and height, and forest sites (Fig. 3; Table S1). It was also shown that the combined application of spectroscopy models of leaf $V_{c,max25}$ and age are sufficient to track lifetime variation in leaf $V_{c,max25}$ (Fig. 4). This represents a significant breakthrough in our ability to rapidly estimate and potentially map $V_{c,max25}$ in high spatial and temporal resolution in tropical forests.

Consistent with previous studies (Field, 1983; Sobrado, 1994; Wilson *et al.*, 2001; Kitajima *et al.*, 2002; Kenzo *et al.*, 2006; Pantin *et al.*, 2012; Albert *et al.*, 2018), large variability in leaf $V_{c,max25}$ (i.e. 7–102 μmol CO_2 m^{-2} s^{-1}) was found with species, leaf age and canopy environment across 21 species sampled from the three lowland moist tropical forests. Such a wide range of variability in $V_{c,max25}$ is comparable with previous studies at the

same forest sites with larger sample size and a focus on mature leaves (i.e. 15–75 μmol CO_2 m^{-2} s^{-1} from 65 species in Panama, Norby *et al.*, 2017; 10–80 μmol CO_2 m^{-2} s^{-1} from 38 species in Brazil, Domingues *et al.*, 2014). It also is comparable with the observations from other moist tropical forest sites in Brazil (Carswell *et al.*, 2000), Peru (Bahar *et al.*, 2017) and Africa (Domingues *et al.*, 2010).

These past studies together with the present findings also suggest that leaf age is one of the most important sources of variation in $V_{c,max25}$, which is clearly shown in Figs 1 and 4. Because $V_{c,max25}$ can change markedly with leaf age and the observed $V_{c,max25}$ variability found herein spanned almost the same range (13–90 μmol CO_2 m^{-2} s^{-1}) as that used to represent global variation in $V_{c,max25}$ in current ESMs (Rogers, 2014), it further suggests the importance of including such age-dependent $V_{c,max25}$ variation in future model formulations. The high $V_{c,max25}$ variability associated with leaf age also highlights the value of the spectroscopy approach developed herein to enable rapid estimations of $V_{c,max25}$: the spectroscopy approach only takes a few seconds to estimate $V_{c,max25}$, once the model has been recalibrated, whereas the conventional approach using leaf gas exchange measurement of a photosynthetic CO_2 response curves takes about an hour (or more). Furthermore, the conventional approach cannot simultaneously derive leaf age, which is an important piece of information needed to improve model representation of photosynthesis, especially in species-rich, evergreen tropical forests (Kim *et al.*, 2012; Wu *et al.*, 2016).

In the present paper it is demonstrated that leaf spectroscopy offers a tool to rapidly capture multiple important axes of variation in $V_{c,max25}$: a single spectra-based model of leaf $V_{c,max25}$ was able to predict leaf $V_{c,max25}$ across tropical tree species with a very large variation in LMA trait space, leaf age, canopy position and forest sites with high confidence (Fig. 3; Table S1). This finding validates pioneering work that not only demonstrates the feasibility of using leaf spectra to model $V_{c,max}$ under a narrow range of species and conditions (Doughty *et al.*, 2011; Serbin *et al.*, 2012), but also dramatically expands our confidence to use the improved spectra–$V_{c,max25}$ model across a wide range of species, leaf developmental stages and locations.

So what is the underlying mechanism for such a tight covariation between leaf spectra and $V_{c,max25}$ across the various axes of variation (i.e. species, leaf age, canopy positions and forest sites) considered in this study? There are at least two potential hypotheses.

The first is that the tightly coordinated variation in leaf $V_{c,max25}$ and spectra was entirely based on their relationships with leaf nitrogen (N) content (Dechant *et al.*, 2017). The theory underlying this hypothesis is that leaf $V_{c,max25}$ is tightly coupled with the N content in Rubisco which comprises the largest fraction of N invested in a single enzyme within a leaf (Jacob *et al.*, 1995; Onoda *et al.*, 2004; Dong *et al.*, 2017; Scafaro *et al.*, 2017; Evans & Clark, 2019). This hypothesis seems moderately supported by two previous studies conducted at the study sites used herein (that focused on mature leaves with larger sample size), including significant, but only modest correlations of $V_{c,max25}$ with leaf N ($R^2 = 0.31$; Norby *et al.*, 2017; $R^2 = 0.33$; Domingues
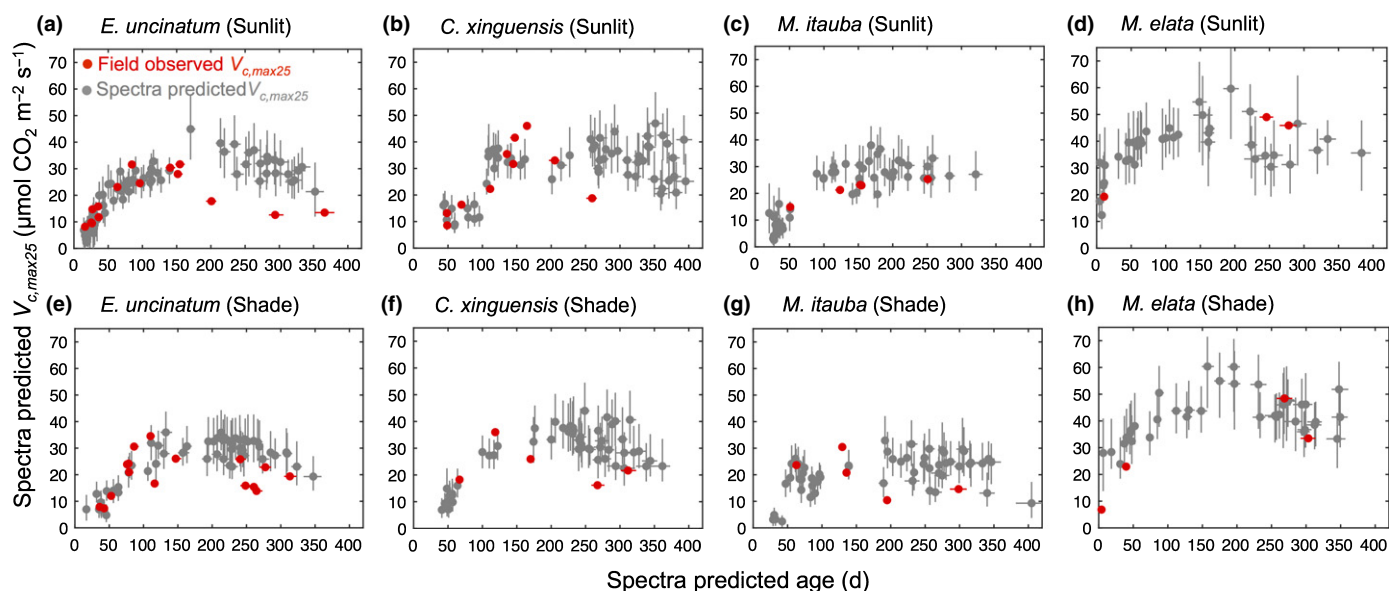
Fig. 4 The combination of the final spectral model for maximum carboxylation rate of Rubisco standardized to 25°C ($V_{c,max25}$) (Fig. 3) and the leaf age model (see Wu *et al.*, 2017; also see the Materials and Methods section) enables the prediction of the life-history trajectories of leaf $V_{c,max25}$ (gray circles) in an independent spectra-age dataset collected in Brazil (see the Materials and Methods section), including four tree-species of (a, e) *E. uncinatum*, (b, f) *C. xinguensis*, (c ,g) *M. itauba*, and (d, h) *M. elata*. The top panels (a, b, c, d) are shown for the sunlit leaves, while the bottom panels (e, f, g, h) are shown for the shaded leaves. Here it is shown that for a given leaf age (determined by the spectra model) it is possible to capture the dynamics of field observed $V_{c,max25}$ (red circles). See Supporting Information Table S1 for the full species names, error bars denote the 95% confidence interval of spectral predictions.

et al., 2014). Given that leaf spectra can efficiently capture variation in leaf N content (Asner & Martin, 2008; Serbin *et al.*, 2014; Dechant *et al.*, 2017), it is expected that leaf spectra also can be used to predict leaf $V_{c,max25}$. However, this hypothesis does not stand up to further scrutiny for several reasons. First, if the correlation with leaf N content was the primary driver of the ability to estimate $V_{c,max25}$ with spectra, it does not explain why leaf spectra show far higher predictive power than using leaf N content alone ($R^2 = 0.89$ for leaf spectra in the present study vs $R^2 = 0.31–0.33$ for leaf nitrogen content as shown in Domingues *et al.*, 2014 and Norby *et al.*, 2017). Furthermore, the $V_{c,max25}$–leaf N relationship does not always hold up at the site level (Bahar *et al.*, 2017; Rogers *et al.*, 2017b; Evans & Clark, 2019) and recent global syntheses have shown that variation in leaf N can only explain a small portion of variation in $V_{c,max}$ (Ali *et al.*, 2015; Smith *et al.*, 2019). Secondarily, many other studies suggest that in addition to leaf N content, other leaf traits, such as leaf phosphorus (P) content (Walker *et al.*, 2014; Norby *et al.*, 2017), leaf chlorophyll content (Croft *et al.*, 2017), LMA (Walker *et al.*, 2014) and age (Albert *et al.*, 2018), also are related to $V_{c,max25}$, and inclusion of more traits as predictive variables can significantly improve the power of trait based model to predict $V_{c,max25}$, compared with the just one trait, leaf N content (Walker *et al.*, 2014). Finally, Serbin *et al.* (2012) showed that in poplar the power of leaf N content and LMA to predict $V_{c,max}$ varied with temperature treatments, but the reflectance spectroscopy approach collapsed this variation into a single model. This suggests that the ability of the spectroscopy approach to predict $V_{c,max}$ is not entirely dependent on the ability of spectroscopy to predict leaf N content and LMA and that other factors are likely contributing to the success of the approach.

Because the correlation with leaf N might not be the only reason for the derived spectra–$V_{c,max25}$ model, this further leads to the second hypothesis: leaf $V_{c,max25}$ is correlated with multiple leaf traits and processes that determine Rubisco content and activity (e.g. leaf N content, leaf P content, leaf Chl concentrations, LMA, leaf age and many others we do not yet understand), and leaf spectra emerge from the ensemble of properties that define leaf chemical, morphological and phenological status (Asner & Martin, 2008; Serbin *et al.*, 2014; Chavana-Bryant *et al.*, 2017, 2019). As such, leaf spectra can be used to help infer leaf $V_{c,max25}$, and are indeed a better predictor of leaf $V_{c,max25}$ (Serbin *et al.*, 2012) than alternative trait approaches that leverage well-established links between $V_{c,max25}$ and just one or a few individual leaf traits (Walker *et al.*, 2014). This second hypothesis offers a more plausible explanation for the power of the spectra–$V_{c,max25}$ approach. However, a more comprehensive study to elucidate the underlying mechanisms that enable the spectra–$V_{c,max25}$ model is still needed.

The finding herein that the spectra–$V_{c,max25}$ model of mature leaves in Panama creates model bias when applied to Panamanian immature leaves and all Brazilian leaves also is interesting. This observation could be attributable to different ranges in $V_{c,max25}$ for model development and validation (e.g. 17–102 μmol CO_2 m^{-2} s^{-1} for the Panamanian mature leaf model; 21–63 μmol CO_2 m^{-2} s^{-1} for Panamanian immature leaves; 7–46 μmol CO_2 m^{-2} s^{-1} for all leaves in Brazil). However, a more likely explanation is that the spectra–trait–$V_{c,max25}$ linkages (e.g. regression coefficients) vary with leaf age (Field, 1983; Wilson *et al.*, 2001; Chavana-Bryant *et al.*, 2017, 2019; Wu *et al.*, 2017) and forest sites of different soil types and fertility (Walker *et al.*, 2014; Norby *et al.*, 2017). Regardless of these potential reasons,

this finding (Fig. 3) suggests that including as many axes of variation as possible in the training dataset is critical to develop a broadly applicable spectra–$V_{c,max25}$ model. Both leaf spectra and $V_{c,max25}$ can vary with vertical canopy profiles (e.g. various canopy positions including upper canopy, mid-canopy and understory trees; sunlit and shaded environments), different tropical forests types (e.g. flooded, Caatinga, second growth and upland forests) and other leaf habits (e.g. deciduous trees) that are currently either under or not sampled in the present study. Therefore, it is apparent that further in-depth pan-tropical and global sampling and analysis are still needed to develop a highly robust spectra–$V_{c,max25}$ model that can be applied with confidence throughout the tropics and ultimately globally. It also is worth noting that when extending the spectra–$V_{c,max25}$ model to entire vertical canopy profiles, the epiphyll effect is another issue that is needed to be considered, as many old leaves in the shaded canopy environment develop epiphylls (Sonnleitner et al., 2009), which strongly impacts leaf spectral reflectance (see Roberts et al., 1998).

It was also shown that spectroscopy is able to simulate the life-history variability in $V_{c,max25}$ with leaf age within and across tropical tree species (Fig. 4). The spectroscopy-derived age-dependent $V_{c,max25}$ also is comparable to direct measurements by Wu et al. (2016) in terms of both amplitude and the relative trend across three leaf developmental stages: young (1–2 months), mature (3–5 months) and old (6–14 months). This suggests that the spectroscopy approach can be used to track lifetime trajectories in leaf traits (including but not limited to $V_{c,max25}$ shown here), and is a marked extension of previous studies that demonstrated the feasibility of linking leaf spectroscopy to model leaf $V_{c,max25}$ (Serbin et al., 2012) or leaf age (Chavana-Bryant et al., 2017). Moreover, the success of spectroscopy-based $V_{c,max25}$–age relationships also highlights that the spectroscopy approach can not only be a novel means to capture the variability of $V_{c,max25}$, particularly associated with leaf age, but also rapidly generate datasets that enable the exploration of temporal and spatial variability in $V_{c,max25}$ within and across species, an important piece of process knowledge that greatly needs to be incorporated in future ESMs (Xu et al., 2017).

Finally, the finding that there exists a tight relationship among leaf-level spectra, $V_{c,max25}$ and leaf age can likely be extended to canopy and ecosystem scales. As shown by many previous theoretical and empirical studies (Asner, 1998; Asner & Martin, 2008; Ollinger, 2011; Singh et al., 2015), the fundamental changes in leaf optical properties with underlying variation in leaf traits is not scale-dependent; that is, leaf-level or canopy-scale spectra change in concert with leaf traits. Meanwhile, Serbin et al. (2015) demonstrated that the imaging spectroscopy technique could be effectively used to infer leaf $V_{c,max25}$ from canopy-scale hyperspectral reflectance for managed agricultural sites. Given the predictive success at the leaf level found herein, it is possible that these findings could help enable better canopy-level predictions in tropical forest ecosystems. Additionally, Wu et al. (2018) connected leaf-scale optical properties (i.e. reflectance and transmittance) with canopy radiative transfer models to simulate the leaf age effect on canopy reflectance in tropical forest ecosystems. The simulated canopy reflectance from the present analysis showed a good agreement with observations from high resolution WorldView-2 imagery, further suggesting a potential way to scale up the leaf-scale spectra–$V_{c,max25}$ relationship explored herein to the canopy scale. Collectively, these recent studies, together with the present findings (Figs 3, 4), help lay down a solid foundation from which to build the possibility of monitoring $V_{c,max25}$–age relationships at canopy and ecosystem scales using state-of-the-art remote sensing technology, for example, leveraging imaging spectroscopy from unmanned aerial systems (UASs; Adão et al., 2017), aircraft (e.g. AVIRIS, Serbin et al., 2015), and the suite of current and planned space-borne platforms (e.g. EnMAP, Guanter et al., 2015; HISUI, Stavros et al., 2017; Surface Biology and Geology mission, National Academies of Sciences E, Medicine, 2018). Imaging spectroscopy, if successful in capturing $V_{c,max25}$–age relationships at canopy scales, will greatly advance our capability to monitor and mechanistically understand $V_{c,max25}$ variability across both space and time, providing critically important datasets to parameterize and evaluate ESMs.

Author contributions

JW, AR, LPA, SRS and SPS designed the study; KE, SPS, AR, BTW and JW collected the field data in Panama; LPA, NP, RCO and JW collected the field data in Brazil; JW performed the data quality control and analysis; JW drafted the manuscript, and all authors contributed to the final version.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Example demonstration of partial least-squares regression (PLSR) analysis for spectra–$V_{c,max25}$ relationship.

Fig. S2 Example demonstration of partial least-squares regression (PLSR) analysis for spectra-age relationship.

Fig. S3 The final spectra–$V_{c,max25}$ model was trained using two-thirds of our entire dataset, and then applied to the remaining, independent validation datasets.

Notes S1. The sample code for a combined, spectral models of leaf $V_{c,max25}$ and age.

Table S1 Site, species, canopy positions, traits and leaf age for trees sampled with leaf spectral and physiological measurements in two Panamanian tropical forests and one Brazilian tropical forest.

Table S2 Leaf gas exchange, spectra, LMA and age data sources.

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